Supplemental Information for

CATALYTIC DETOXIFICATION OF NERVE AGENT AND PESTICIDE ORGANOPHOSPHATES BY BUTYRYLCHOLINESTERASE ASSISTED WITH NON-PYRIDINIUM OXIMES

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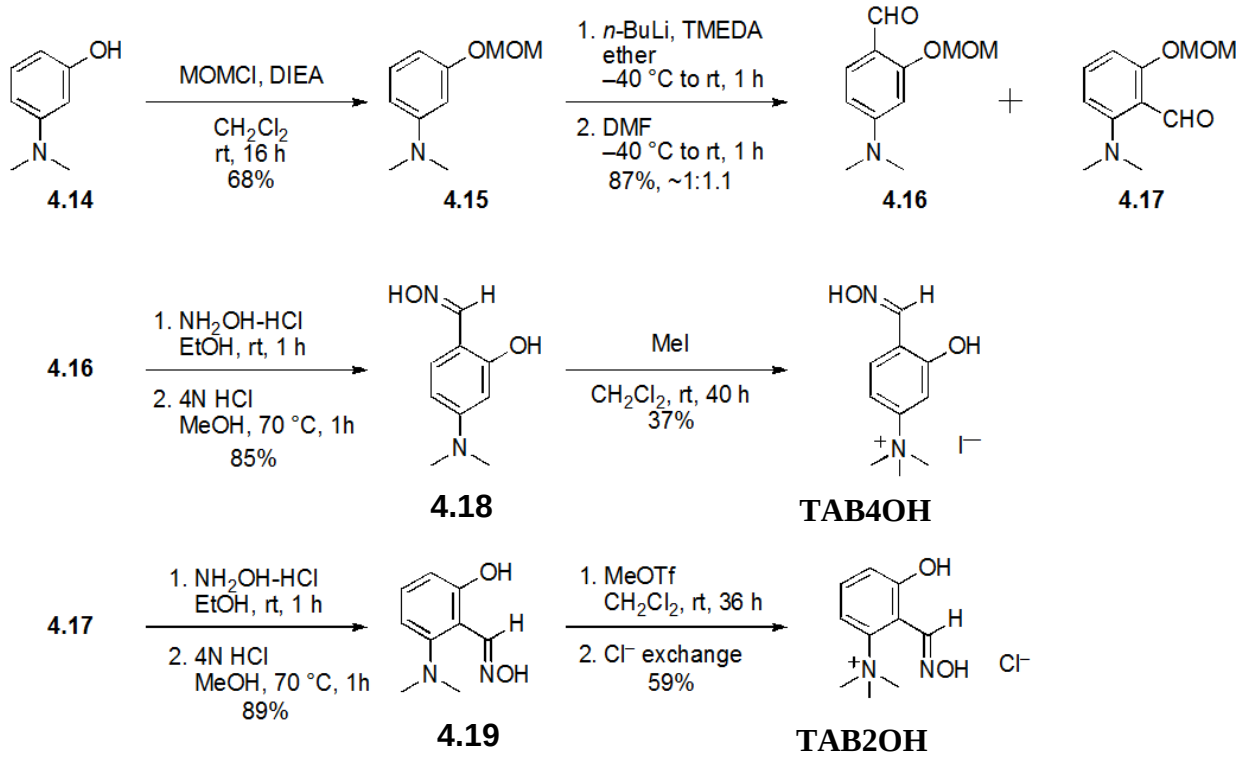
Table of Contents

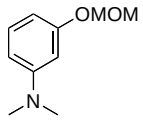
General Information

^{1}H NMR and ^{13}C NMR spectra were recorded at 600 MHz respectively, using a Bruker DRX-600 spectrometer equipped with a 5 mm QNP probe. Chemical shifts of ^{1}H NMR and ^{13}C NMR are given in ppm by using deuterated solvents as references. Standard abbreviations indicating multiplicity were used as follows: s (singlet), br (broad), d (doublet), t (triplet), q (quartet), m (multiplet). MALDI-TOF spectra and high-resolution mass spectra (HRMS) were recorded on an Applied Biosystems Voyager STR (2) apparatus and an Agilent ESI-TOF mass spectrometer respectively. Anhydrous CH2Cl2, NEt3 and Et2O were taken from a solvent drying system (SG Water USA).

Synthetic procedures:

Scheme S1: Synthesis of TAB2OH and TAB4OH.





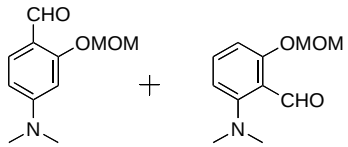
(3-Methoxymethoxy-phenyl)-dimethylamine **4.15**

To a stirring solution of 3-dimethylaminophenol (1.2 g, 8.8 mmol) in CH_2Cl_2 (12 mL) at 0 °C was added DIEA (3.0 mL, 17.2 mmol) and chloromethyl methyl ether (1.2 mL, 15.8 mmol) and the solution was stirred at room temperature for 3 h. The reaction was quenched with a saturated solution of $NaHCO_3$ and stirred for 30 min. The layers were separated and the aquous layer extracted twice with CH_2Cl_2, the fractions combined, dried with $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude material was purified twice by flash chromatography eluting once with CH_2Cl_2 and the second time with 9:1 hexanes:EtOAc to afford 660 mg of pure product as a colorless oil. (42%).

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.13-7.16 (m, 1H), 6.40-6.44 (m, 3H), 5.17 (s, 2H), 3.49 (s, 3H), 2.94 (s, 6H).

^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 158.4, 152.0, 129.7, 106.7, 103.9, 101.1, 94.5, 55.9, 40.5.

HRMS (MH^+) expected: 182.1175; found: 182.1179.



2-Dimethylamino-6-methoxymethoxy-benzaldehyde and 4-Dimethylamino-2-methoxymethoxy-benzaldehyde **4.16** and **4.17**

To a stirring solution of **4.15** (600 mg, 3.3 mmol) and freshly distilled *N,N,N',N'*-tetramethylethylenediamine (0.5 mL, 3.3 mmol) in dry ethyl ether (10 mL) cooled to –40 °C under argon was added *n*-butyllithium (2.0 mL of 1.8 M in hexanes solution, 3.6 mmol) and the reaction was allowed to warm to room temperature and stirred for 1 h. The reaction was re-cooled to –40 °C and dry DMF (0.5 mL, 6.5 mmol) was added and the reaction was allowed to warm to room temperature with stirring over 1 h before being quenched with a dilute NH_4Cl aqueous solution. The mixture was extracted 3 times with EtOAc, the organic fractions combined, dried over $MgSO_4$, filtered, and then the volatiles were removed under reduced pressure. The crude material was purified by flash chromatography over silica gel eluting with 4:1 hexanes:EtOAc to separate the isomers and then each one was individually purified by flash flash chromatography over silica gel eluting with CH_2Cl_2 to afford each isomer pure.

4-Dimethylamino-2-methoxymethoxy-benzaldehyde **4.16**

281 mg, pale yellow solid, 41%.

^{1}H-NMR (600 MHz, $CDCl_3$) δ 10.19 (s, 1H), 7.73 (d, J = 8.9 Hz, 1H), 6.36 (dd, J = 8.9, 2.0 Hz, 1H), 6.33 (d, J = 2.0 Hz, 1H), 5.28 (s, 2H), 3.52 (s, 3H), 3.06 (s, 6H).

^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 187.4, 161.8, 155.8, 130.1, 115.2, 105.7, 96.3, 94.7, 56.4, 40.1.

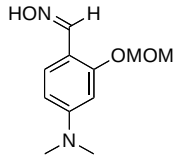
HRMS (MH^+) expected: 210.1125; found: 210.1125.

2-Dimethylamino-6-methoxymethoxy-benzaldehyde **4.17**

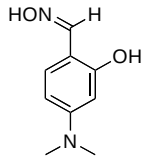
317 mg, yellow oil, 46%.

^{1}H-NMR (600 MHz, $CDCl_3$) δ 10.37 (s, 1H), 7.32 (t, J = 8.3 Hz, 1H), 6.63 (d, J = 8.3 Hz, 2H), 5.25 (s, 2H), 3.51 (s, 3H), 2.89 (s, 6H).

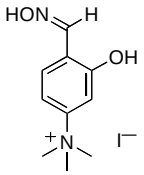
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 188.6, 161.1, 155.2, 134.9, 115.8, 110.4, 105.1, 95.0, 56.5, 44.7.
HRMS (MH^+) expected: 210.1125; found: 210.1123.



4-Dimethylamino-2-methoxymethoxy-benzaldehyde oxime
To a stirring solution of **4.16** (130 mg, 0.62 mmol) in EtOH (10 mL) was added hydroxylamine hydrochloride (77 mg, 1.1 mmol) and the reaction was stirred at room temperature for 30 min. A saturated solution of $NaHCO_3$ and EtOAc were added and the layers were separated. The aqueous layer was extracted twice with EtOAc, the organic fractions combined, dried over $MgSO_4$, filtered, and then the volatiles were removed under reduced pressure. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography over silica gel eluting with 1:3:1 hexanes:CH_2Cl_2:EtOAc to afford 89 mg of pure product as a white solid. (64%)
21 mg of a second product was isolated that appears to be the oxime isomer (15%).
^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.78 (bs, 1H), 7.57 (d, J = 8.8 Hz, 1H), 6.43 (d, J = 2.4 Hz, 1H), 6.33 (dd, J = 8.8, 2.4 Hz, 1H), 5.22 (s, 2H), 3.50 (s, 3H), 2.99 (s, 6H).
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 156.9, 152.8, 146.9, 127.5, 109.4, 106.3, 98.1, 94.8, 56.2, 40.3.
HRMS (MH^+) expected: 225.1234; found: 225.1233.



4-Dimethylamino-2-hydroxy-benzaldehyde oxime **4.18**
To a stirring solution of 4-dimethylamino-2-methoxymethoxy-benzaldehyde oxime (80 mg, 0.36 mmol) in MeOH (2 mL) at room temperature was added a 4 M solution of HCl in dioxane (0.3 mL, 1.2 mmol). The reaction was heated to 70 °C for 1 h and then cooled to room temperature and the solvent was blown off with nitrogen. The crude material was purified by flash chromatography over silica gel eluting with CH_2Cl_2 to afford 50 mg of pure product as a white solid. (82%)
^{1}H-NMR (600 MHz, $CDCl_3$) δ 9.83 (s, 1H), 8.11 (s, 1H), 7.00 (d, J = 8.2 Hz, 1H), 6.93 (s, 1H), 6.27 (m, 2H), 2.99 (s, 6H).
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 158.7, 153.1, 152.7, 131.7, 105.5, 104.2, 99.0, 40.1.
HRMS (MH^+) expected: 181.0971; found: 181.0969.



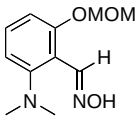
4-Trimethylanilinium-2-hydroxy-benzaldehyde oxime iodide **TAB4OH**

To a stirring solution of **4.18** (32 mg, 0.18 mmol) in CH_2Cl_2 (0.7 mL) at room temperature was added iodomethane (0.1 mL, 1.6 mmol). The reaction was stirred at room temperature for 40 h as a precipitate formed. The product was collected by filtration washing with excess CH_2Cl_2 yielding 21 mg of pure product as a white solid. (37%)

^{1}H-NMR (600 MHz, CD_3OD) δ 8.36 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 1H), 7.47 (d, *J* = 2.7 Hz, 1H), 7.44 (dd, *J* = 8.7, 2.7 Hz, 1H), 3.69 (s, 9H).

^{13}C-NMR (150.9 MHz, CD_3OD) δ 159.5, 150.2, 149.5, 133.6, 121.0, 111.8, 109.6, 57.7.

HRMS (M^+) expected: 195.1133; found: 195.1133.



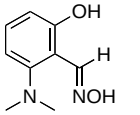
2-Dimethylamino-6-methoxymethoxy-benzaldehyde oxime

To a stirring solution of **4.17** (163 mg, 0.78 mmol) in EtOH (10 mL) was added hydroxylamine hydrochloride (85 mg, 1.2 mmol) and the reaction was stirred at room temperature for 30 min. A saturated solution of $NaHCO_3$ and EtOAc were added and the layers were separated. The aqueous layer was extracted twice with EtOAc, the organic fractions combined, dried over $MgSO_4$, filtered, and then the volatiles were removed under reduced pressure. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography over silica gel eluting with 1:3:1 hexanes:CH_2Cl_2:EtOAc to afford 160 mg of pure product as a white solid. (91%)

^{1}H-NMR (600 MHz, $CDCl_3$) δ 9.93 (s, 1H), 8.46 (s, 1H), 7.24 (t, *J* = 8.2 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.76 (d, *J* = 8.2 Hz, 1H), 5.25 (s, 2H), 3.50 (s, 3H), 2.75 (s, 6H).

^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 156.4, 154.8, 146.0, 130.4, 114.5, 112.1, 108.9, 94.7, 56.3, 44.9.

HRMS (MH^+) expected: 225.1234; found: 225.1234.



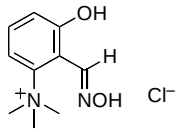
2-Dimethylamino-6-hydroxy-benzaldehyde oxime **4.19**

To a stirring solution of 2-dimethylamino-6-methoxymethoxy-benzaldehyde oxime (14 mg, 0.06 mmol) in MeOH (0.2 mL) at room temperature was added a 4 M solution of HCl in dioxane (0.2 mL, 0.8 mmol). The reaction was heated to 70 °C for 1 h and then cooled to room temperature and the solvent was blown off with nitrogen. The crude material was purified by flash chromatography over silica gel eluting with CH_2Cl_2 to afford 10 mg of pure product as a white solid. (91%)

^{1}H-NMR (600 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.65 (s, 1H), 7.21 (t, *J* = 8.1 Hz, 1H), 7.11 (s, 1H), 6.66 (d, *J* = 8.2 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 2.73 (s, 6H).

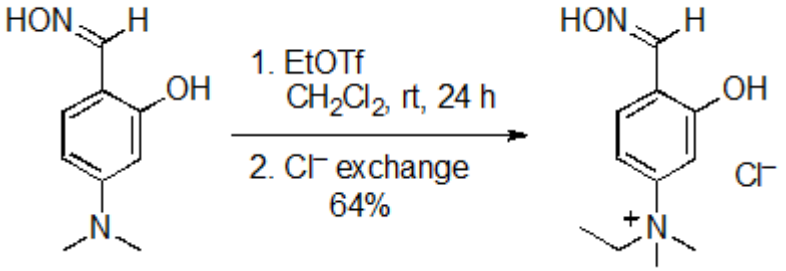
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 158.5, 154.8, 151.4, 131.6, 111.3, 110.2, 109.8, 45.6.

HRMS (MH^+) expected: 181.0971; found: 181.0983.

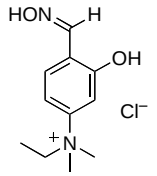


2-Trimethylanilinium-6-hydroxy-benzaldehyde oxime chloride **TAB2OH**
To a stirring solution of **4.19** (90 mg, 0.50 mmol) in CH_2Cl_2 (0.2 mL) at 0 °C was added MeOTf (62 μL, 0.55 mmol). The reaction was allowed to warm to room temperature and stirred for 40 h. The precipitate formed was collected by filtration washing with excess CH_2Cl_2 to afford 101 mg of the pure triflate salt as a white solid. (59%) The salt was dissolved in CH_3CN and a solution of tetrahexylammonium chloride in CH_3CN was added to precipitate the pure chloride salt as a white solid.
^{1}H-NMR (600 MHz, d_6-DMSO) δ 11.74 (s, 1H), 10.95 (s, 1H), 8.34 (s, 1H), 7.45 (t, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 3.68 (s, 9H).
^{13}C-NMR (150.9 MHz, d_6-DMSO) δ 158.9, 145.6, 145.5, 130.6, 117.5, 113.5, 111.3, 57.2.
HRMS (M^+) expected: 195.1133; found: 195.1130.

Scheme S2: Synthesis of TAB4OHmme.



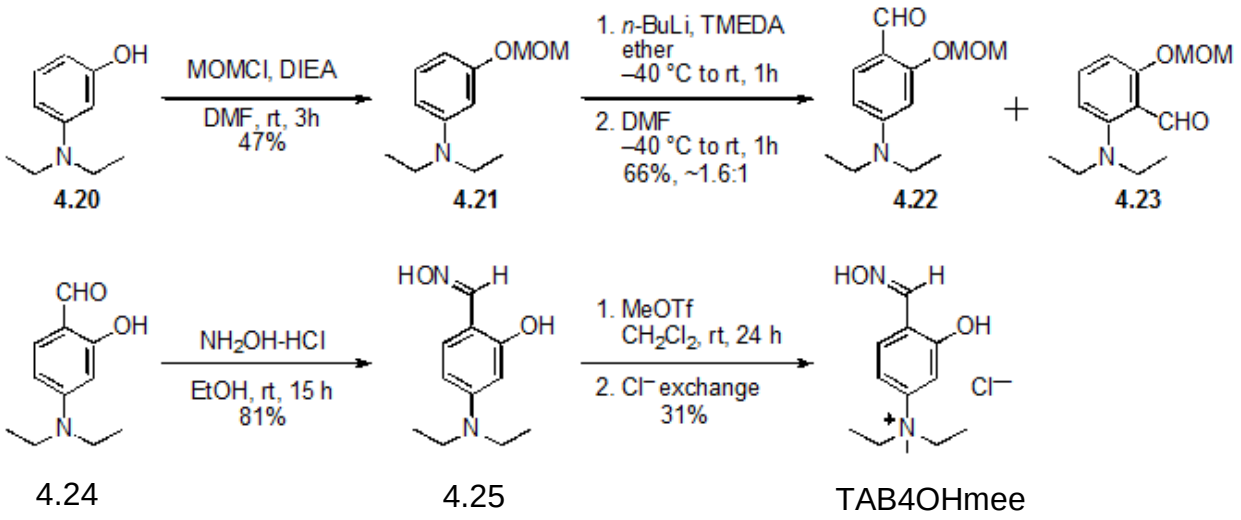
4.18 TAB4OHmme

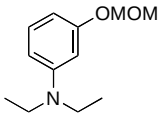


4-Dimethylethylanilinium-2-hydroxy-benzaldehyde oxime chloride **TAB4OHmme,**
To a stirring solution of **4.18** (53 mg, 0.29 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added EtOTf (42 μL, 0.32 mmol). The reaction was allowed to warm to room temperature and stirred for 24 h. The mixture was tritrated with CH_2Cl_2 and then the triflate salt was dissolved in CH_3CN and a solution of tetrahexylammonium chloride in CH_3CN was added to precipitate 46 mg of the pure chloride salt as a white solid. (64%)
^{1}H-NMR (600 MHz, d_6-DMSO) δ 11.72 (s, 1H), 10.94 (s, 1H), 8.37 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.44 (m, 1H), 7.39 (dd, *J* = 8.8, 2.6 Hz, 1H), 3.90 (q, *J* = 7.2 Hz, 2H), 3.53 (s, 6H), 1.00 (t, *J* = 7.2 Hz, 3H).

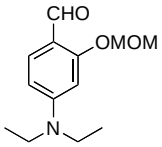
^{13}C-NMR (150.9 MHz, d_6-DMSO) δ 156.5, 145.7, 145.0, 128.8, 119.9, 112.0, 109.4, 63.8, 53.0, 8.4.
HRMS (M^+) expected: 209.1284; found: 209.1294.

Scheme S3: Synthesis of TAB4OHmee.



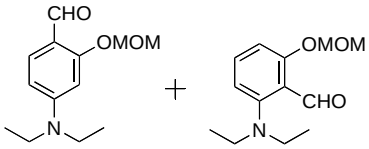


(3-Methoxymethoxyphenyl)-diethylamine **4.21**
To a stirring solution of 3-diethylaminophenol (1.29 g, 7.8 mmol) in CH_2Cl_2 (12 mL) at 0 °C was added DIEA (2.0 mL, 11.5 mmol) and chloromethyl methyl ether (0.75 mL, 9.9 mmol) and the solution was stirred at room temperature for 15 h. The reaction was quenched with a 10% solution of NaOH and stirred for 30 min. The layers were separated and the aquous layer extracted twice with CH_2Cl_2, the fractions combined, dried with $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude material was purified by flash chromatography eluting with 9:1 hexanes:EtOAc to afford 900 mg of pure product as a colorless oil. (55%).
^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.09-7.12 (m, 1H), 6.35-6.36 (m, 3H), 5.16 (s, 2H), 3.49 (s, 3H), 3.34 (q, J = 7.1 Hz, 4H) 1.16 (t, J = 7.1 Hz, 6H).
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 158.6, 149.2, 129.9, 106.0, 102.6, 100.2, 94.5, 55.9, 44.4, 12.6.
HRMS (MH^+) expected: 210.1488; found: 210.1492.



4-Diethylamino-2-methoxymethoxy-benzaldehyde **4.22**
To a stirring solution of 4-diethylaminosalicylaldehyde (436 mg, 2.3 mmol) in DMF (6 mL) at 0 °C was added DIEA (0.6 mL, 3.5 mmol) and chloromethyl methyl ether (0.2 mL, 2.6 mmol) and

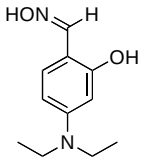
the solution was stirred at room temperature for 3 h. The reaction was quenched with a 5% NaOH solution and stirred for 30 min. EtOAc was added and the layers were separated and the aquous layer extracted twice with EtOAc, the fractions combined, dried with $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude material was purified twice by flash chromatography eluting once with CH_2Cl_2 and the second time with 9:1 CH_2Cl_2:EtOAc to afford 250 mg of pure product. (47%).
^{1}H-NMR (600 MHz, $CDCl_3$) δ 10.16 (s, 1H). 7.71 (d, *J* = 9.0 Hz, 1H), 6.33-6.35 (m, 2H), 5.26 (s, 2H), 3.52 (s, 3H), 3.41 (q, *J* = 7.1 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H).
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 187.1, 162.1, 153.7, 130.3, 114.7, 105.4, 95.9, 94.7, 56.3, 44.8, 12.5.
HRMS (MH^+) expected: 238.1438; found: 238.1438.



4-diethylamino-2-methoxymethoxy-benzaldehyde and 2-Diethylamino-6-methoxymethoxy-benzaldehyde **4.22** and **4.23**
To a stirring solution of **4.21** (840 mg, 4.0 mmol) and freshly distilled *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (0.61 mL, 4.1 mmol) in dry ethyl ether (15 mL) cooled to –40 °C under argon was added *n*-butyllithium (2.5 mL of 1.8 M in hexanes solution, 4.5 mmol) and the reaction was allowed to warm to room temperature and stirred for 1 h. The reaction was re-cooled to –40 °C and dry DMF (0.6 mL, 7.7 mmol) was added and the reaction was allowed to warm to room temperature with stirring over 1 h before being quenched with a dilute NH_4Cl aqueous solution. The mixture was extracted 3 times with EtOAc, the organic fractions combined, dried over $MgSO_4$, filtered, and then the volatiles were removed under reduced pressure. The crude material was purified by flash chromatography over silica gel eluting with CH_2Cl_2. The isomers were separated by eluting with 7:3 hexanes:EtOAc to afford each isomer pure.

4-Diethylamino-2-methoxymethoxy-benzaldehyde **4.22**
386 mg, pale yellow oil, 41%.
^{1}H-NMR (600 MHz, $CDCl_3$) δ 10.16 (s, 1H). 7.72 (d, *J* = 8.8 Hz, 1H), 6.33-6.35 (m, 2H), 5.26 (s, 2H), 3.52 (s, 3H), 3.41 (q, *J* = 7.1 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H).
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 187.1, 162.1, 153.7, 130.3, 114.7, 105.4, 95.9, 94.7, 56.3, 44.8, 12.6.
HRMS (MH^+) expected: 238.1438; found: 238.1438.

2-Diethylamino-6-methoxymethoxy-benzaldehyde **4.23**
239 mg, yellow oil, 25%.
^{1}H-NMR (600 MHz, $CDCl_3$) δ 10.25 (s, 1H). 7.35 (t, *J* = 8.3 Hz, 1H), 6.77 (dd, *J* = 8.3, 2.3 Hz, 2H), 5.25 (s, 2H), 3.51 (s, 3H), 3.19 (q, *J* = 7.1 Hz, 4H), 1.07 (t, *J* = 7.1 Hz, 6H).
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 190.1, 159.5, 155.0, 134.1, 120.3, 114.5, 108.1, 95.1, 56.5, 48.2, 12.3.
HRMS (MH^+) expected: 238.1438; found: 238.1440.



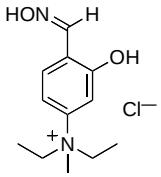
4-Diethylamino-2-hydroxy-benzaldehyde oxime **4.25**

To a stirring solution of 4-diethylamino-salicylaldehyde (494 mg, 2.6 mmol) in EtOH (10 mL) was added hydroxylamine hydrochloride (220 mg, 3.2 mmol) and the reaction was stirred at room temperature for 15 h. A saturated solution of $NaHCO_3$ and EtOAc were added and the layers were separated. The aqueous layer was extracted twice with EtOAc, the organic fractions combined, dried over $MgSO_4$, filtered, and then the volatiles were removed under reduced pressure. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography over silica gel eluting with CH_2Cl_2 to afford 432 mg of pure product as an off-white solid. (81%)

^{1}H-NMR (600 MHz, $CDCl_3$) δ 9.79 (s, 1H), 8.09 (s, 1H), 6.96 (m, 1H), 6.83 (bs, 1H), 6.22-6.24 (m, 2H), 3.36 (q, *J* = 7.1 Hz, 4H), 1.18 (t, *J* = 7.1 Hz, 6H).

^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 159.0, 153.1, 150.3, 131.9, 104.7, 103.7, 98.2, 44.4, 12.6.

HRMS (MH^+) expected: 209.1284; found: 209.1285.



4-Diethylmethylanilinium-2-hydroxy-benzaldehyde oxime chloride TAB4OHmee,

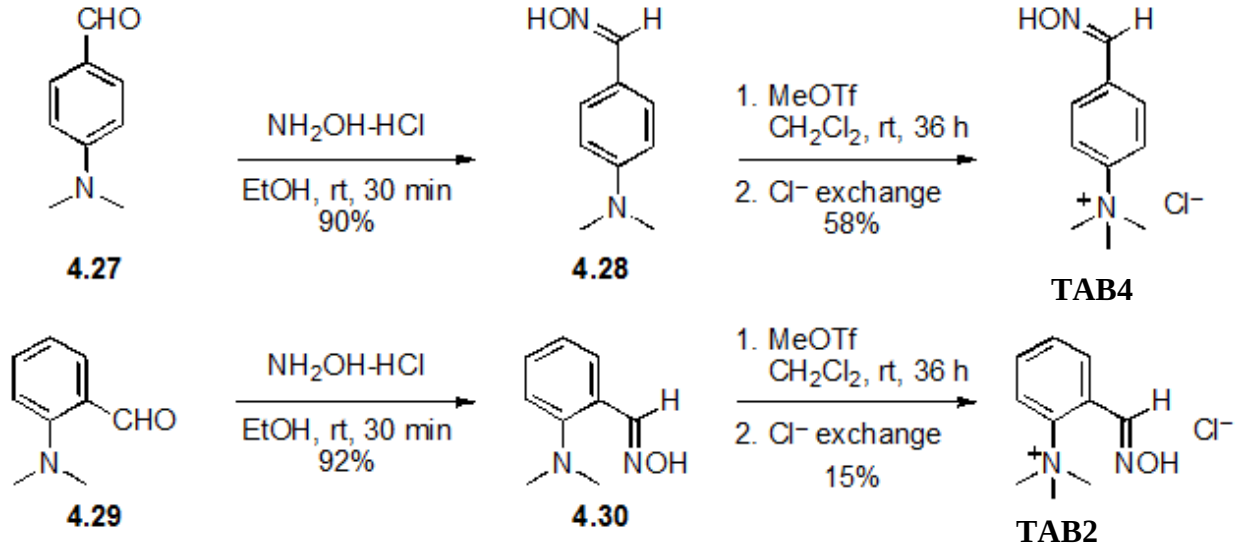
To a stirring solution of **4.25** (98 mg, 0.47 mmol) in CH_2Cl_2 (0.7 mL) at 0 °C was added MeOTf (62 μL, 0.55 mmol). The reaction was allowed to warm to room temperature and stirred for 23 h. The volatiles were then blown off with nitrogen and the residue purified twice by flash chromatography over silica gel by eluting with a gradient of 19:1 to 17:1 CH_2Cl_2:MeOH to afford the pure tosylate salt. The salt was dissoved in MeOH and eluted through DOWEX 1-2x200 resin to exchange for the chloride anion and the material was recrystallized from EtOH to afford 38 mg of the pure chloride salt as a white solid. (31%)

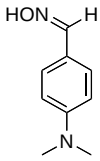
^{1}H-NMR (600 MHz, CD_3OD) δ 8.37 (s, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 7.33 (d, *J* = 2.7 Hz, 1H), 7.29 (dd, *J* = 8.7, 2.7 Hz, 1H), 4.06 (m, 2H), 3.86 (m, 2H), 3.52 (s, 3H), 1.19 (t, *J* = 7.1 Hz, 6H).

^{13}C-NMR (150.9 MHz, CD_3OD) δ 159.7, 150.2, 143.4, 132.7, 121.0, 113.7, 111.4, 65.6, 46.7, 8.8.

HRMS (M^+) expected: 223.1446; found: 223.1446.

Scheme S4: Synthesis of TAB2 and TAB4.





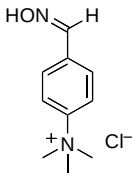
4-Dimethylamino-benzaldehyde oxime **4.28**

To a stirring solution of 4-dimethylamino-benzaldehyde (457 mg, 3.1 mmol) in EtOH (17 mL) was added hydroxylamine hydrochloride (290 mg, 4.2 mmol) and the reaction was stirred at room temperature for 15 h as a yellow color developed. A saturated solution of $NaHCO_3$ and EtOAc were added and the layers were separated. The aqueous layer was extracted twice with EtOAc, the organic fractions combined, dried over $MgSO_4$, filtered, and then the volatiles were removed under reduced pressure. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography over silica gel eluting with a gradient of 1:0 to 4:1 CH_2Cl_2:EtOAc to afford 470 mg of pure product as a white solid. (93%)

^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.45 (d, J = 8.9 Hz, 2H), 6.69 (d, J = 8.9, 2H), 3.00 (s, 9H).

^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 151.5, 150.6, 128.3, 119.7, 111.9, 40.2.

HRMS (MH^+) expected: 165.1022; found: 165.1028.



4-Trimethylanilinium-benzaldehyde oxime chloride of **TAB4.**

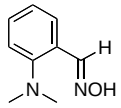
To a stirring solution of **4.28** (75 mg, 0.46 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added MeOTf (57 μL, 0.50 mmol). The reaction was allowed to warm to room temperature and stirred for 19 h.

The formed precipitate was collected by filtration washing with excess CH_2Cl_2 to afford 128 mg of the pure triflate salt as a white solid. (85%) The salt was dissolved in CH_3CN and a solution of tetrahexylammonium chloride in CH_3CN was added to precipitate the pure chloride salt as a white solid. (58%)

^{1}H-NMR (600 MHz, d_6-DMSO) δ 11.64 (s, 1H), 8.24 (s, 1H), 8.03 (d, *J* = 9.1 Hz, 2H), 7.81 (d, *J* = 9.1, 2H), 3.64 (s, 9H).

^{13}C-NMR (150.9 MHz, d_6-DMSO) δ 147.3, 146.4, 134.6, 127.4, 121.0, 56.2.

HRMS (M^+) expected: 179.1179; found: 179.1180.



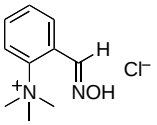
2-Dimethylamino-benzaldehyde oxime **4.30**

To a stirring solution of 2-dimethylamino-benzaldehyde (266 mg, 1.8 mmol) in EtOH (5 mL) was added hydroxylamine hydrochloride (137 mg, 2.0 mmol) and the reaction was stirred at room temperature for 30 min as the yellow color faded to colorless. A saturated solution of $NaHCO_3$ and EtOAc were added and the layers were separated. The aqueous layer was extracted twice with EtOAc, the organic fractions combined, dried over $MgSO_4$, filtered, and then the volatiles were removed under reduced pressure. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography over silica gel eluting with 2:2:1 hexanes:CH_2Cl_2:EtOAc to afford 270 mg of pure product as a white solid. (92%)

^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.61 (bs, 1H), 8.47 (s, 1H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.34 (t, *J* = 8.5 Hz, 1H), 7.07 (d, *J* = 8.1 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 2.75 (s, 6H).

^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 153.1, 149.0, 130.5, 127.5, 125.4, 122.5, 118.5, 45.2.

HRMS (MH^+) expected: 165.1022; found: 165.1025.



2-Trimethylanilinium-benzaldehyde oxime chloride of **TAB2**.

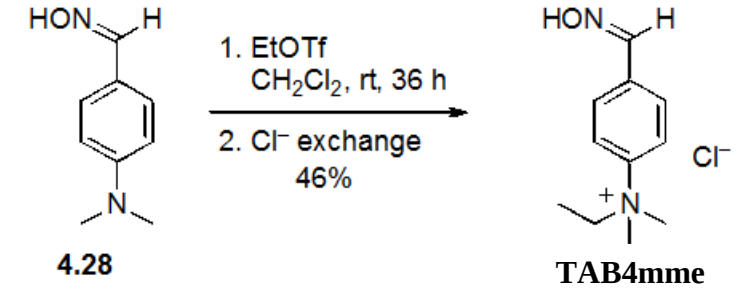
To a stirring solution of **4.30** (69 mg, 0.42 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added MeOTf (50 μL, 0.44 mmol). The reaction was allowed to warm to room temperature and stirred for 36 h. After the solvent was blown off under nitrogen, the salt was dissoved in MeOH and eluted through DOWEX 1-2x200 resin to exchange for the chloride anion. After drying under reduced pressure, the crude material was dissolved in a mixture of EtOAc and water and the layers were separated. The organic layer was extracted once with water and then the aqueous solution was lyophilized. The material was then tritrated with CH_2Cl_2 and CH_3CN to afford 16 mg of a white solid that was 85% pure, a mixture of the chloride salt with an unknown impurity. (18%)

^{1}H-NMR (600 MHz, CD_3OD) δ 8.75 (s, 1H), 7.99 (d, *J* = 7.7 Hz, 1H), 7.77 (t, *J* = 8.5 Hz, 1H), 7.68 (m, 2H), 3.80 (s, 9H).

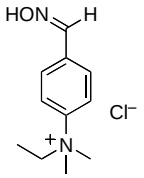
^{13}C-NMR (150.9 MHz, CD_3OD) δ 148.4, 146.5, 135.1, 132.3, 132.0, 128.2, 122.3, 58.5.

HRMS (M^+) expected: 179.1179; found: 179.1182.

Scheme S5: Synthesis of TAB4mme.

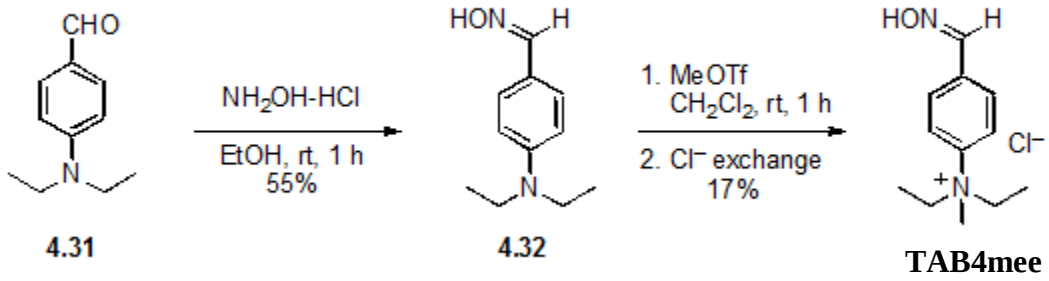


4.28 **TAB4mme**

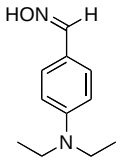


4-Dimethylethylanilinium-benzaldehyde oxime chloride **TAB4mme.**
To a stirring solution of **4.28** (66 mg, 0.40 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added EtOTf (57 μL, 0.44 mmol). The reaction was allowed to warm to room temperature and stirred for 24 h. The formed precipitate was collected by filtration washing with excess CH_2Cl_2 to afford 90 mg of the pure triflate salt. (65%) The salt was dissolved in CH_3CN and a solution of tetrahexylammonium chloride in CH_3CN was added to precipitate 42 mg of the pure chloride salt as a white solid. (46%)
^{1}H-NMR (600 MHz, d_6-DMSO) δ 11.63 (s, 1H), 8.24 (s, 1H), 7.94 (d, *J* = 9.0 Hz, 2H), 7.82 (d, *J* = 9.0, 2H), 3.96 (q, *J* = 7.2 Hz, 2H), 3.59 (s, 6H), 0.99 (t, *J* = 7.2 Hz, 3H).
^{13}C-NMR (150.9 MHz, d_6-DMSO) δ 146.4, 144.2, 134.6, 127.5, 121.8, 63.9, 53.1, 8.5.
HRMS (M^+) expected: 193.1335; found: 193.1335.

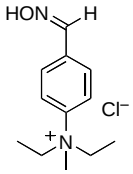
Scheme S6: Synthesis of TAB4mee.



4.31 **4.32** **TAB4mee**

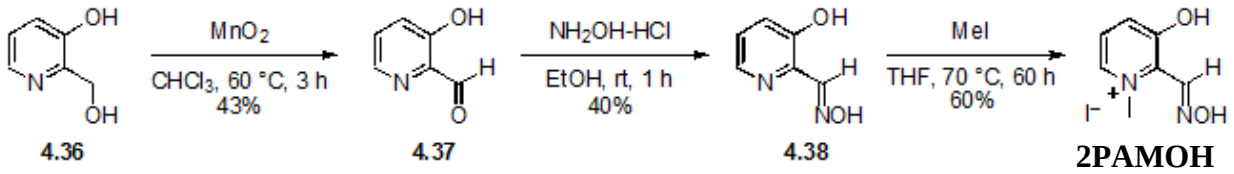


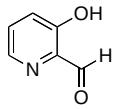
4-Diethylamino benzaldehyde oxime **4.32**
To a stirring solution of 4-diethylamino-benzaldehyde (417 mg, 2.4 mmol) in EtOH (5 mL) was added hydroxylamine hydrochloride (171 mg, 2.5 mmol) and the reaction was stirred at room temperature for 1 h. A saturated solution of $NaHCO_3$ and EtOAc were added and the layers were separated. The aqueous layer was extracted twice with EtOAc, the organic fractions combined, dried over $MgSO_4$, filtered, and then the volatiles were removed under reduced pressure. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography over silica gel eluting with a gradient of 3:1 to 2:1 hexanes:EtOAc to afford 250 mg of pure product as an off-white solid. (55%)
^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.79 (bs, 1H), 7.42 (d, *J* = 8.7 Hz, 1H), 6.65 (bd, *J* = 7.3 Hz, 1H), 3.38 (q, *J* = 7.1 Hz, 4H), 1.18 (t, *J* = 7.1 Hz, 6H).
^{13}C-NMR (150.9 MHz, $CDCl_3$) δ 150.5, 149.0, 128.5, 118.6, 111.3, 44.4, 12.5.
HRMS (MH^+) expected: 193.1341; found: 193.1334.



4-Diethylmethylanilinium-benzaldehyde oxime chloride **TAB4mee**.
To a stirring solution of **4.32** (71 mg, 0.37 mmol) in CH_2Cl_2 (1 mL) at 0 °C was added MeOTf (50 μL, 0.44 mmol). The reaction was allowed to warm to room temperature and stirred for 24 h. After the solvent was blown off under nitrogen, the salt was dissoved in MeOH and eluted through DOWEX 1-2x200 resin to exchange for the chloride anion and the material was tritrated with CH_2Cl_2 to afford 15 mg of the pure chloride salt as a white solid. (17%)
^{1}H-NMR (600 MHz, CD_3OD) δ 8.17 (s, 1H), 7.88 (d, *J* = 9.1 Hz, 1H), 7.78 (d, *J* = 9.1 Hz, 1H), 4.09 (m, 2H), 3.87 (m, 2H), 3.54 (s, 3H), 1.15 (t, *J* = 7.2 Hz, 6H).
^{13}C-NMR (150.9 MHz, CD_3OD) δ 147.7, 142.6, 137.1, 129.6, 123.5, 65.6, 46.8, 8.9.
HRMS (M^+) expected: 207.1492; found: 207.1492.

Scheme S7: Synthesis of 2PAMOH.





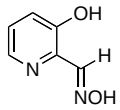
3-Hydroxypyridine-2-carboxaldehyde **4.37**

A suspension of 2-(hydroxylmethyl)-3-hydroxy-pyridine (1.0 g, 8.0 mmol) in $CHCl_3$ (20mL) was added to a stirring suspension of MnO_2 (4.0 g) in $CHCl_3$ (20 mL) at 50 °C. The reaction was heated to 60 °C for 3 h and then the suspension was filtered through celite washing with excess $CHCl_3$. The solvent was removed under reduced pressure to afford 430 mg of pure product. (43%)

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.77 (s, 1H), 10.10 (s, 1H), 8.30 (d, J = 4.3 Hz, 1H), 7.55 (dd, J = 8.5, 4.2 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H).

^{13}C-NMR (150.9 MHz, d_6-DMSO) δ 193.9, 156.8, 141.6, 137.9, 129.7, 125.8.

HRMS (MH^+) expected: 124.0393; found: 124.0395.



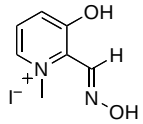
3-Hydroxypyridine-2-carboxaldehyde oxime **4.38**

To a stirring solution of **4.37** (225 mg, 1.8 mmol) in EtOH (10 mL) was added hydroxylamine hydrochloride (137 mg, 2.0 mmol) and the reaction was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography over silica gel eluting with 40:60:0.5 hexanes:EtOAc:TEA to afford 105 mg of product as a white solid. (41%) An analytically pure sample was repurified by flash chromatography over silica gel eluting with 19:1 CH_2Cl_2:MeOH.

^{1}H-NMR (600 MHz, d_6-DMSO) δ 11.87 (s, 1H), 10.29 (s, 1H), 8.31 (s, 1H), 8.15 (dd, J = 4.3, 1.2 Hz, 1H), 7.34 (dd, J = 8.3, 1.1 Hz, 1H), 7.28 (dd, J = 8.3, 4.4 Hz, 1H).

^{13}C-NMR (150.9 MHz, d_6-DMSO) δ 153.1, 150.6, 140.9, 136.6, 124.7, 123.4.

HRMS (MH^+) expected: 139.0502; found: 139.0503.



3-Hydroxy-2-pyridinealdoxime methiodide **2PAMOH**

To a stirring solution of **4.38** in THF (2 mL) was added iodomethane (0.1 mL, 1.6 mmol) and the reaction was heated to 70 °C in a sealed tube for 60 h. After cooling, the precipitate was collected by filtration to yield 29 mg of pure product as a pale yellow solid. (60%)

^{1}H-NMR (600 MHz, d_6-DMSO) δ 8.77 (s, 1H), 8.42 (d, J = 5.5 Hz, 1H), 7.99 (d, J = 8.6 Hz, 1H), 7.81 (m, 1H).

^{13}C-NMR (150.9 MHz, d_6-DMSO) δ 159.3, 143.4, 139.7, 134.8, 133.4, 128.4.

HRMS (M^+) expected: 153.0659; found: 153.0664.

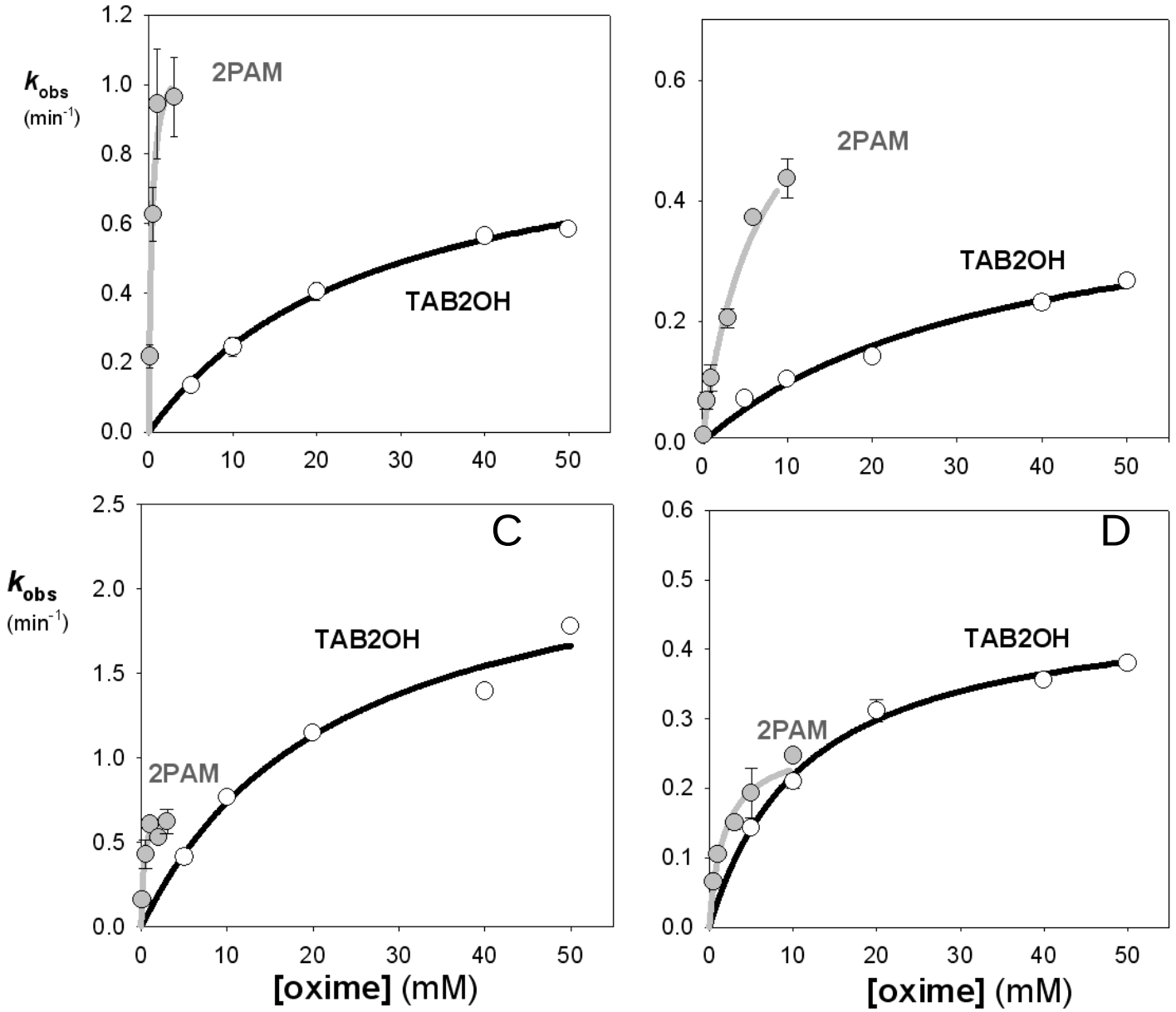


Figure S1. Concentration dependence of oxime reactivation of **A)** sarin, **B)** cyclosarin, **C)** VX and **D)** paraoxon inhibited (conjugated) hAChE. Dependence for the lead oxime **TAB2OH** compared to reference cationic oxime 2PAM (measured at 37 ^{o}C in 0.10M phosphate buffer pH 7.4). Data from two to four experiments are shown with associated SE of determination. For TAB2OH errors were smaller than symbol size.

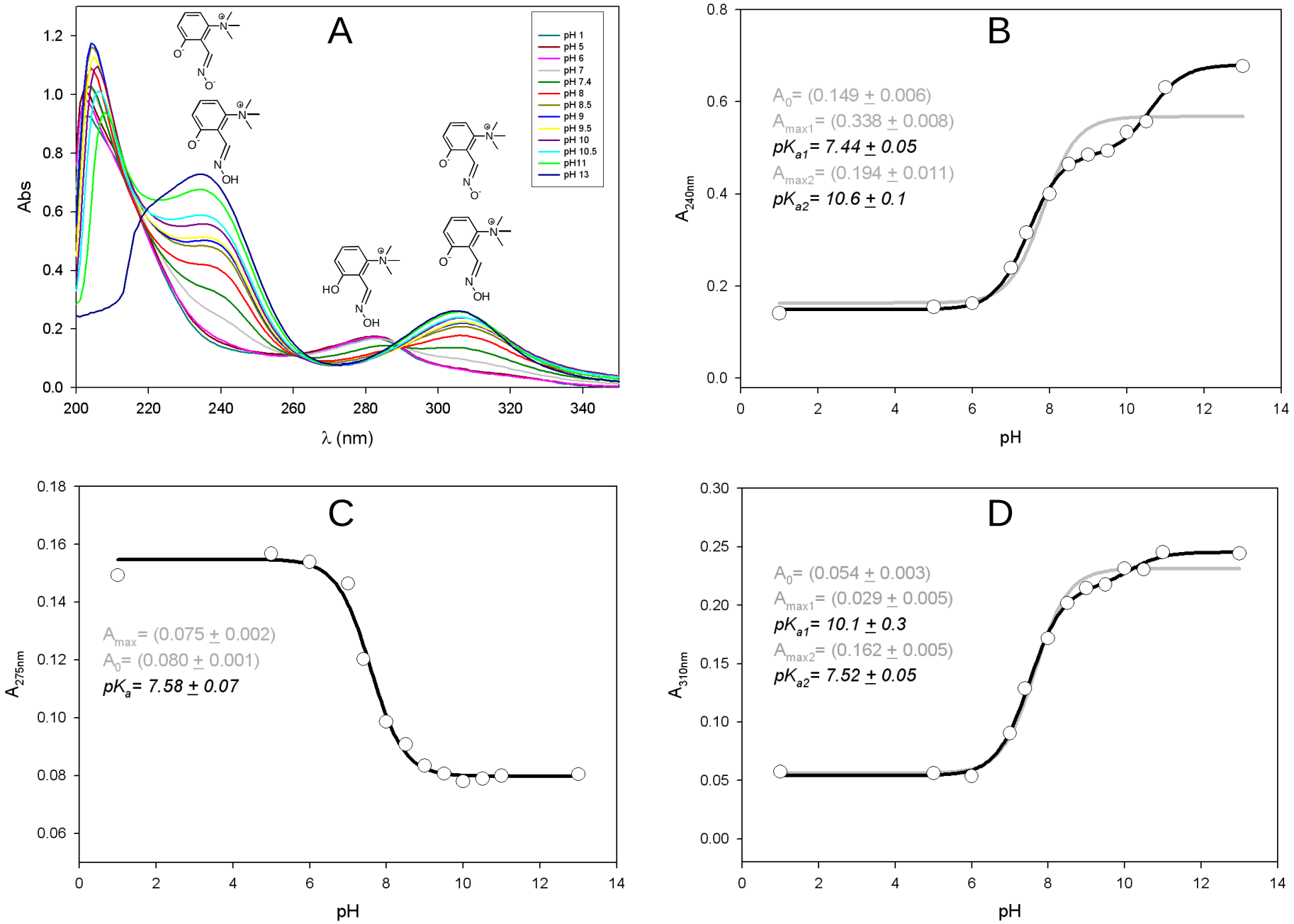


Figure S2. pH dependence of **A)** UV spectra of 50 µM **TAB2OH** and pH dependences of **B)** A_{240nm} of 50 µM **TAB2OH**, **C)** A_{275nm} of 50 µM **TAB2OH**, and **D)** A_{310nm} of 50 µM **TAB2OH**, along with corresponding pK_a values calculated by nonlinear regression of equation 2 (black curves in B and D) or equation 1 (grey curves in B and D) [16].

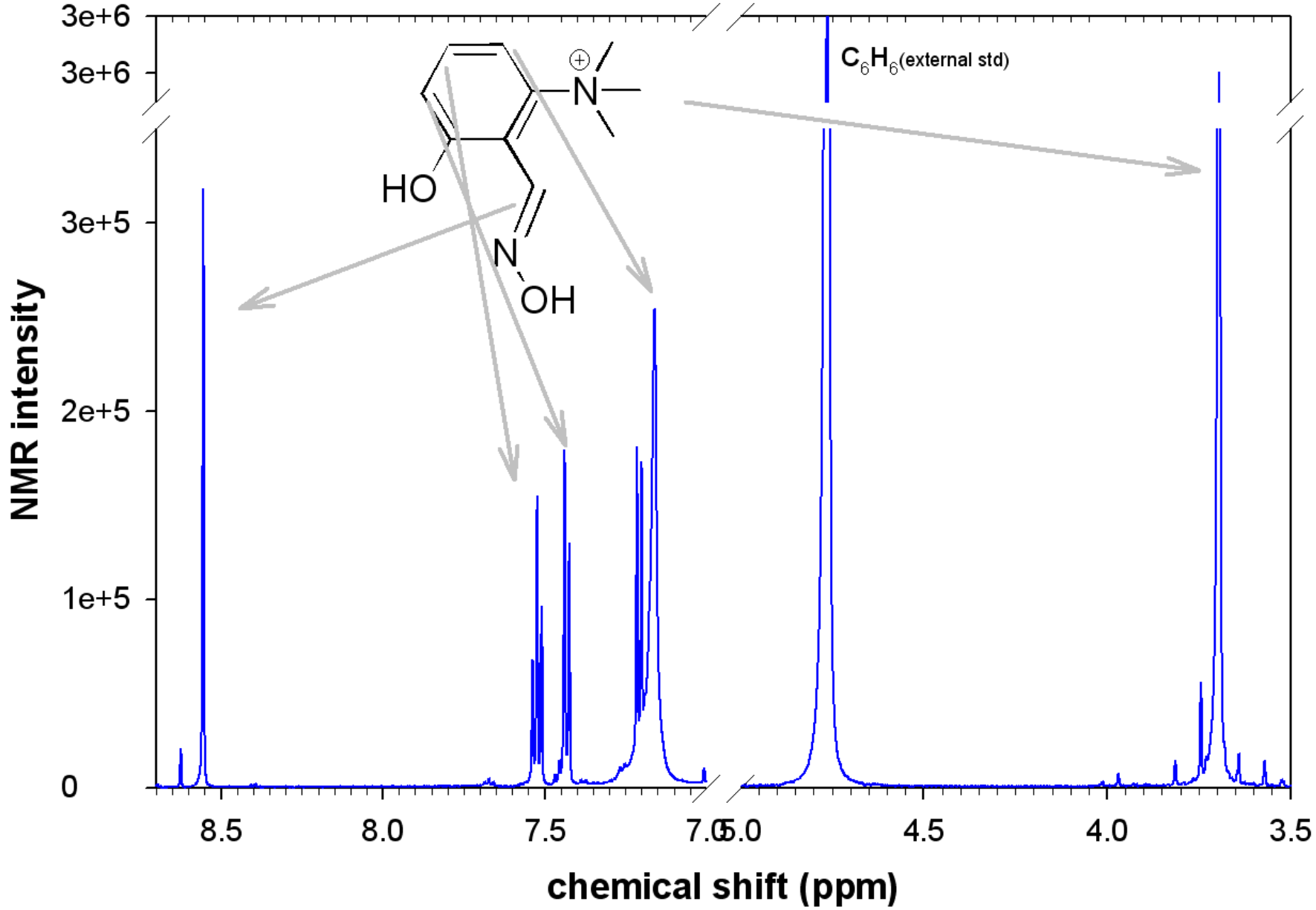


Figure S3. Peak assignment in the ^{1}H NMR spectrum of 10 mM **TAB2OH** in 20 mM phosphate-pyrophosphate (+0.1M NaCl) D_2O buffer pH 5.

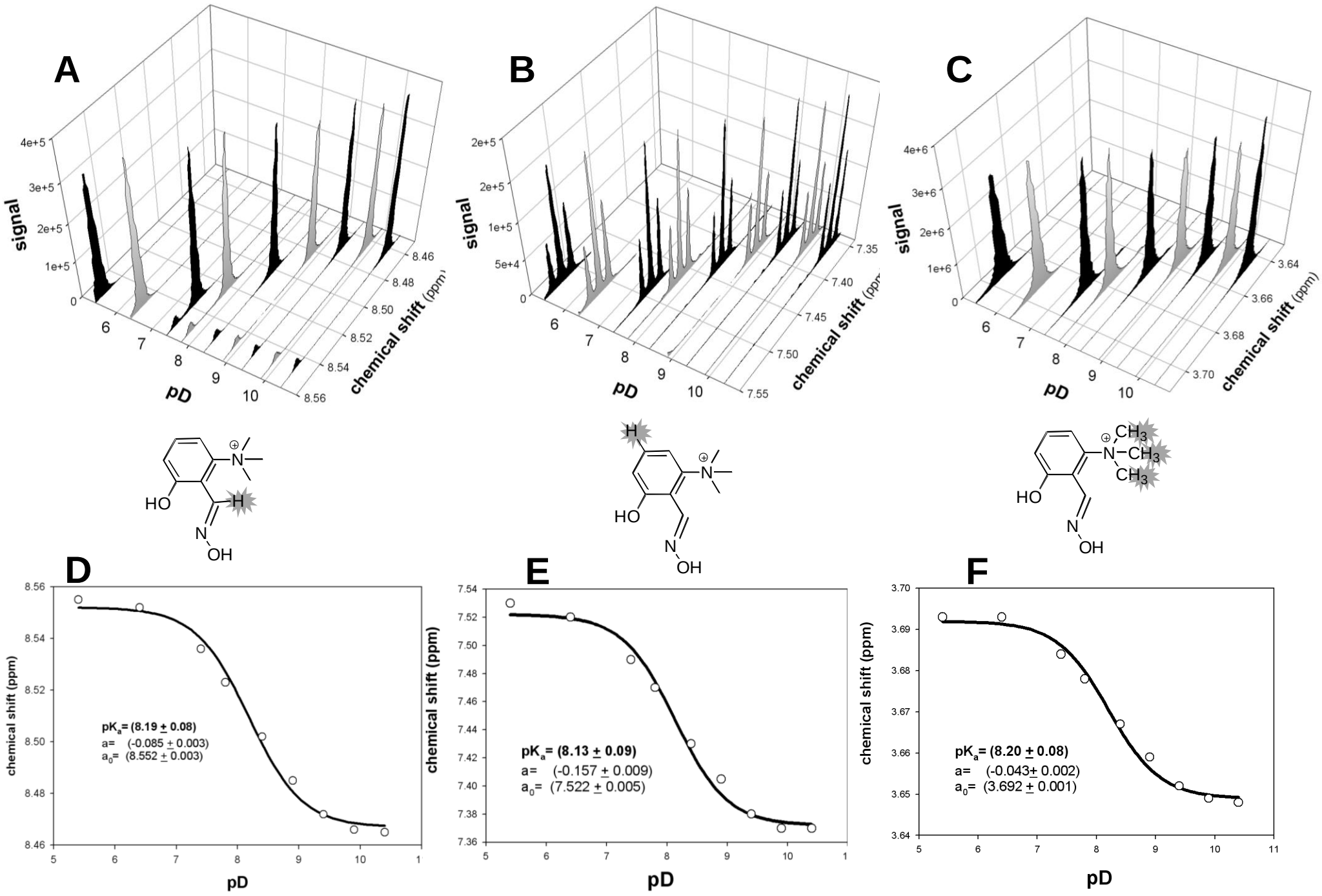


Figure S4. pH dependence of ^{1}H NMR spectra of 10 mM **TAB2OH** in D_2O buffers (**A, B** and **C**) along with corresponding pK_a values calculated from the observed pH induced difference in chemical shifts (**D**, **E** and **F**) by nonlinear regression [16]. Spectra from the single experiment were aligned using benzene external standard singlet at 4.55 ppm. Resonating protons are highlighted in **TAB2OH** structure for each of the peaks.

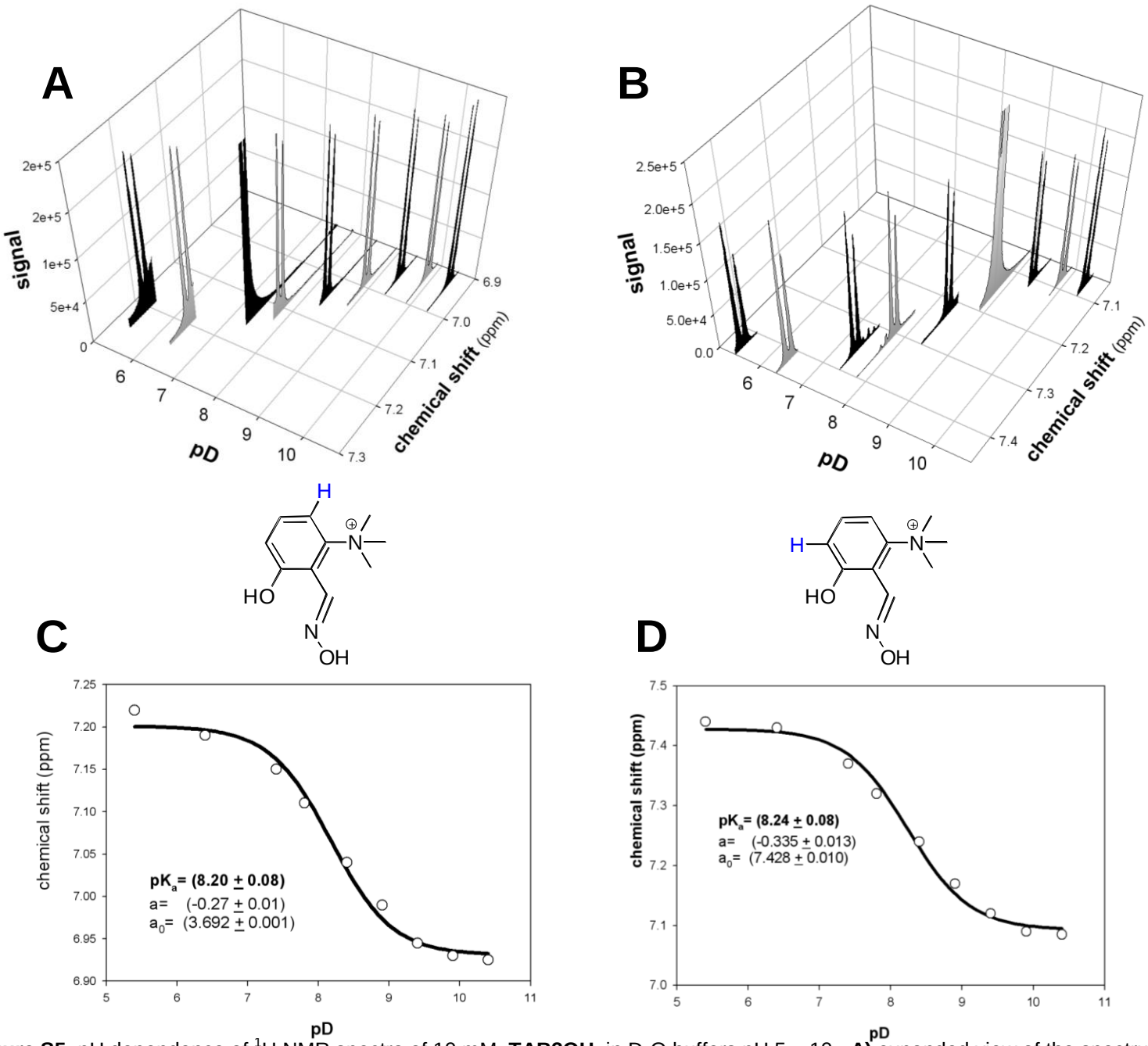


Figure S5. pH dependence of ^{1}H NMR spectra of 10 mM **TAB2OH** in D_2O buffers pH 5 – 10. **A)** expanded view of the spectrum in the chemical shift region 6.9 – 7.3. along with pH dependent change in chemical shifts for the 7.2 ppm doublet and **B)** expanded view of the spectrum in the chemical shift region 7.0 – 7.5. along with pH dependent change in chemical shifts for the 7.42 ppm doublet. The corresponding pK_a values were calculated from the observed pH induced difference in chemical shifts (**C** and **D**) by nonlinear regression [16]. Spectra from the single experiment were aligned using benzene external standard singlet at 4.55 ppm.

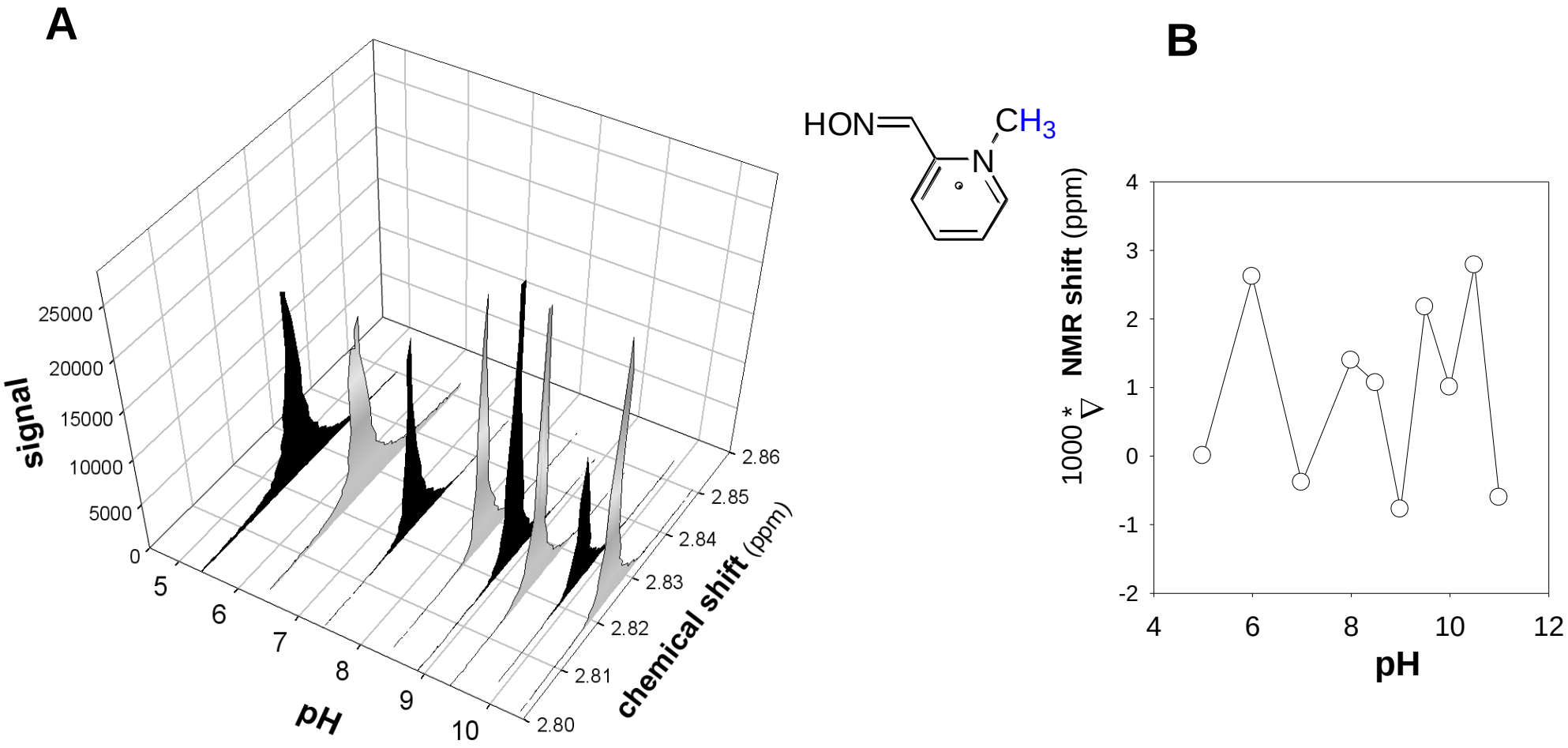


Figure S6. pH dependence of ^{1}H NMR spectra of 2.0 mM 2PAM in D_2O buffers pH 5 – 10. **A)** expanded view of the spectrum in the chemical shift region 2.80 – 2.86. **B)** pH dependent change in chemical shifts for the 2.83 ppm singlet . Spectra from the single experiment were aligned using benzene external standard singlet at 7.16 ppm.

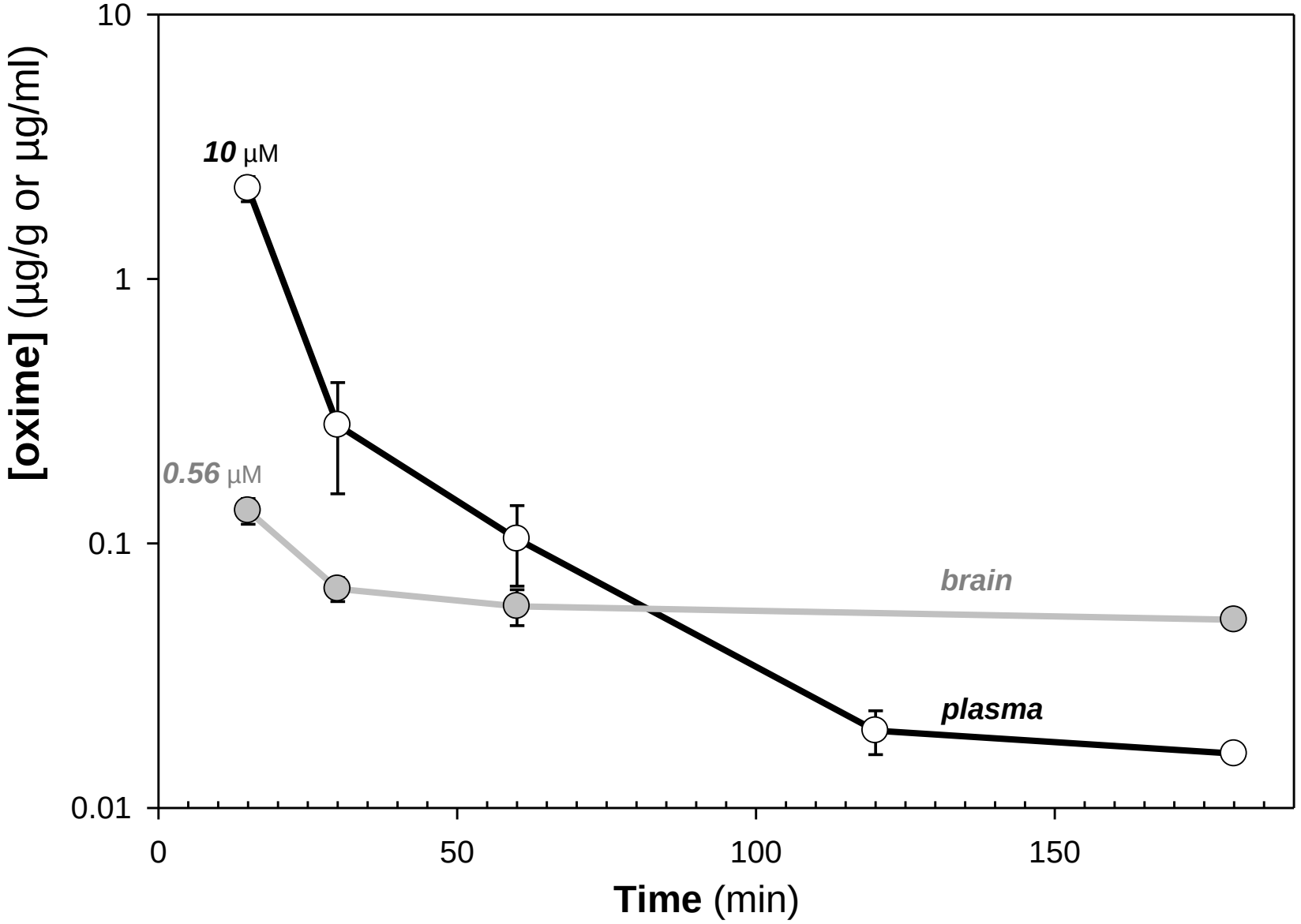


Figure S7. Pharmacokinetics of TAB2OH in mice. Brain (*gray symbols and lines*) and plasma (*white symbols and black lines*) compound concentrations were determined at discrete time points upon single, 30 mg/kg dose administered to mice i.m. Each point represents average of determinations from three mice. S.D. of determination are indicated by *error bars*.

Table S1. Maximal oxime concentrations in brains and plasma of mice determined upon i.m. administration of a single oxime dose (cf. Figure S5). Distribution coefficients (log D) were calculated from oxime structures using ChemAxon software package (www.chemaxon.com).

oxime	**i.m. dose** (mg/kg)	**[oxime]$_{max}$**					**log D**
		(µg/ml)		(µM)			
		brain	**plasma**	**brain**	**plasma**	**brain/plasma**	
TAB2OH	**30**	**0.13**	**2.2**	**0.56**	**10**	**0.056**	**- 2.0**

Table S2. The first order rate constants ***k*** (10^{-5} s^{-1}) for hydrolysis of 5.0 uM nerve agent OP analogues (Flu-MPs) by oximes alone or by combination of 500 nM hBChE and an oxime. Constants refer to the fast phase of hydrolysis shown in Figure 5, representing degradation of a fraction of total racemic OP. Constants calculated in representative experiments are given. The experiments were replicated at least once.

Nerve agent **OP** analogue	**oxime** (1.0mM) **+ OP**		**[BuChE+oxime** (1.0mM)**] + OP**		**oxime** (0.1mM) **+ OP**		**[BuChE + oxime** (0.1mM)**] + OP**	
	TAB2OH	2PAM	TAB2OH	2PAM	TAB2OH	2PAM	TAB2OH	2PAM
sarin	5.5	11	**15**	**27**	4.1	4.6	**8.5**	**12**
cyclosarin	6.4	11	**530** **(30% total OP)**	**37**	3.4	3.7	**180**	**37**
VX	15	28	**430** **(50% total OP)**	**64**	11	13	**150**	**40**

Table S3. Antidotal efficacy of oxime TAB2OH and human BChE in VX, paraoxon or sarin exposed mice. BChE (1 mg/kg) and oxime (25 mg/kg) were administered *i.v.* 15 min prior to OP (*s.c.*). Oxime (25 mg/kg) in therapy was administered *i.m.* together with atropine (10 mg/kg) 1 min after OP exposure.

PRETREATMENT 15 min before OP & THERAPY 1 min after OP exposure						
n x LD_{50}	**VX** (*s.c.* LD_{50}=28.3 μg/kg)		**Paraoxon** (*s.c.* LD_{50}=740.8 μg/kg)		**Sarin** (*s.c.* LD_{50}=238.3 μg/kg)	
	Survived/ treated	Symptoms	Survived/ treated	Symptoms	Survived/ treated	Symptoms
1.0	4 / 4	No visible symptoms.				
1.26	4 / 4	One mice had light tremor during first 10 min upon application.				
1.59	4 / 4	Light tremor 3-4 min upon application.				
2.0	4 / 4	Tremor and light salivation 20 min upon application.			4 / 4	Tremor 20 min upon application. Surviving animals were weak after 24 h.
2.52	4 / 4	Strong tremor, light salivation and respiratory disturbance. All symptoms disappeared after 2 h.			3 / 4	Light tremor 3-4 min upon application. Surviving animals were weak after 24 h.
3.18	4 / 4	Strong tremor, light salivation and respiratory disturbance. All symptoms disappeared after 2 h.			2 / 4	Strong tremor immediately upon application. Surviving animals were weak and apathic after 24 h.
4.0	3 / 4	Strong tremor and salivation 3 min upon application. Mice were weak with respiratory disturbance. Surviving animals were weak after 24 h.			2 / 4	Strong tremor immediately upon application. Surviving animals were weak and apathic after 24 h.
5.0	2 / 4	Strong tremor and respiratory disturbance immediately upon application. Surviving animals were weak after 24 h.			2 / 4	Cramps and strong tremor immediately upon application. Surviving animals were very weak after 24 h.
6.3	1 / 4	Strong tremor, salivation and respiratory disturbance immediately upon application. Surviving mouse was weak after 24 h.			0 / 4	Cramps and strong tremor immediately upon application. Animals died between 5 and 60 min after application.
7.9	2 / 4	Strong tremor, paralysis. Surviving animals were weak after 24 h.				
12.6			4 / 4	Lacrimation, dyspnoea, tremor, edema of the eyelids.		
15.9			1 / 4	Strong tremor, lacrimation, dyspnoea. Surviving mouse was weak after 24 h.		
20.0			1 / 4	3 mice died 3-4 min upon application. Surviving mouse was very weak, had respiratory disturbance and closed eyes after 24h.		
25.2			0 / 4	Strong tremor immediately upon application. Animals died upon 2-3 min upon application		